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Differential Fermentation of Cellulose Allomorphs by Ruminal Cellulolytic Bacteria

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In addition to its usual native crystalline form (cellulose I), cellulose can exist in a variety of alternative crystalline forms (allomorphs) which differ in their unit cell dimensions, chain packing schemes, and hydrogen bonding relationships. We prepared, by various chemical treatments, four different alternative allomorphs, along with an amorphous (noncrystalline) cellulose which retained its original molecular weight. We then examined the kinetics of degradation of these materials by two species of ruminal bacteria and by inocula from two bovine rumens. *Ruminococcus flavefaciens* FD-1 and *Fibrobacter succinogenes* S85 were similar to one another in their relative rates of digestion of the different celluloses, which proceeded in the following order: amorphous > III_I > IV_I > III_{II} > I > II. Unlike *F. succinogenes*, *R. flavefaciens* did not degrade cellulose II, even after an incubation of 3 weeks. Comparisons of the structural features of these allomorphs with their digestion kinetics suggest that degradation is enhanced by skewing of adjacent sheets in the microfibril, but is inhibited by intersheet hydrogen bonding and by antiparallelism in adjacent sheets. Mixed microflora from the bovine rumens showed in vitro digestion rates quite different from one another and from those of both of the two pure bacterial cultures, suggesting that *R. flavefaciens* and *F. succinogenes* (purportedly among the most active of the cellulolytic bacteria in the rumen) either behave differently in the ruminal ecosystem from the way they do in pure culture or did not play a major role in cellulose digestion in these ruminal samples.

Cellulose is one of the most abundant biopolymers on earth and is the chief structural component of plant cell wall materials. It thus is a major contributor to the global carbon cycle, and its biodegradation supports a large number of specialist microorganisms. Research on cellulose biodegradation has been heavily oriented toward studies on the physiological characteristics of cellulolytic microorganisms and the biochemical properties of their cellulolytic enzymes. Although cellulose is arranged in variable and often highly complex ways within plant material, relatively little attention has been paid to the relationship between this "fine structure" of cellulose and its biodegradation. It does appear that fine-structural features such as crystallinity, surface area, and pore structure have variable effects on the rate or extent of biodegradation by different classes of cellulolytic microbes (4).

One structural feature of cellulose that has not been examined systematically for its effect on biodegradation is the variety of physical structures taken by cellulose molecules in their different crystalline forms. The six described allomorphs of cellulose (designated I, II, III_I, III_{II}, IV_I, and IV_{II}) are thought to vary with respect to a number of structural features, including the dimensions of the unit cells which constitute the crystallite, the degree of intrachain and interchain hydrogen bonding within the unit cell, and the polarity of adjacent cellulose sheets within the crystallite (15, 21). Because the crystalline regions of natural celluloses are almost exclusively cellulose I, the alternative allomorphs may in a sense be regarded as substrate analogs and thus may be useful materials for probing the mechanism of cellulose hydrolysis at the molecular level. The alternative allomorphs may also be encountered by cellulolytic microflora used in biomass conversion schemes, since treatments such as strong alkali (18, 19) or liquid ammonia (26), which enhance the digestibility of lignocellulosic materials primarily by delignifying the substrates, are known to cause conversion of cellulose I to other allomorphs.

There is some evidence that the geometry of the crystalline lattice is an important determinant in cellulose biodegradation. Chanzy et al. (3) have shown by electron microscopy that the major cellobiohydrolase of the fungus *Trichoderma reesei* binds to *Valonia* cellulose along a discrete crystallographic plane, and Henrissat et al. (10) have used molecular modeling studies of the cellulose fiber to identify which particular glycosidic linkages on the crystallite surface are the most likely sites of hydrolysis by this enzyme. Kudo et al. (13) have reported that whole cells of the ruminal bacterium *Fibrobacter succinogenes* (whose cellulases are thought to be primarily cell bound [9]) show ordered attachment to cellulose fibers which result in the formation of parallel grooves on the fiber surface, while two species of *Ruminococcus* (whose cellulases are primarily extracellular) show a much more random attachment. Rautela and King (20) examined the rate of degradation of cellulose I, cellulose II, and a mixture of celluloses II and IV by the fungus *T. viride* and noted that growth of the fungus on each preparation resulted in production of a cellulase complex which exhibited the greatest activity on that particular material. Weimer et al. (32) reported that a mixed ruminal inoculum from an alfalfa-fed cow (in which the forage contained cellulose I) fermented cellulose I approximately twice as rapidly as cellulose II during growth in an in vitro digestion system.

To assess in more detail the effect of polymorphism on the fermentability of cellulose, we prepared five different allomorphs of cellulose, examined their fine-structural features, and compared the fermentation kinetics of each allomorph

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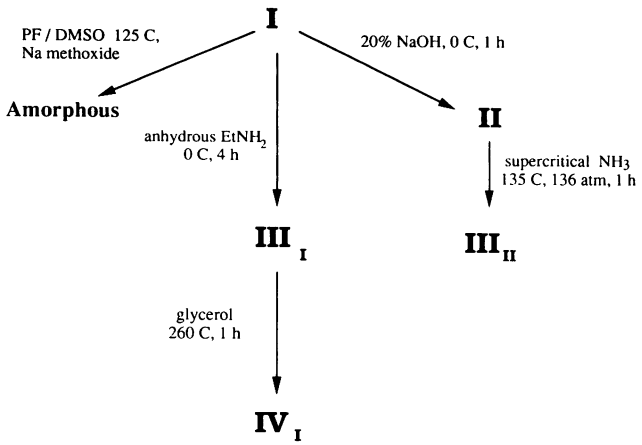

FIG. 1. General preparation scheme for the cellulose allomorphs used in this study. PF/DMSO, paraformaldehyde/dimethyl sulfoxide. See text for specific preparation methods.

by pure cultures of two ruminal cellulolytic bacteria and by mixed ruminal microflora in an in vitro assay system. The kinetic data have permitted an analysis of the relative importance of different internal structural parameters on the fermentation of these substrates.

MATERIALS AND METHODS

Cultures. *F. succinogenes* subsp. *succinogenes* S85 (formerly *Bacteroides succinogenes*; see reference 17) was generously provided by C. W. Forsberg, University of Guelph. *Ruminococcus flavefaciens* FD-1 was a gift from D. M. Schaefer, University of Wisconsin-Madison. Both organisms were grown at a dilution rate of 0.07 h^{-1} in cellulose-limited chemostat culture (33) at 39°C under a CO_2 gas phase on modified Dehority's medium (24) lacking casein hydrolysate and cellobiose, but supplemented with 4.8 g of Sigmacell 20 microcrystalline cellulose (Sigma Chemical Co., St. Louis, Mo.) and 25 ml of sterile clarified ruminal fluid per liter of medium. For mixed-culture fermentations, the ruminal microflora inocula were prepared as described previously (32) from ruminal samples collected from two different cows; one cow (no. 507-J) was a nonlactating Jersey maintained on a diet of 100% alfalfa hay, and the other (no. 748) was a lactating Holstein fed a mixed ration of alfalfa silage, corn silage, corn grain, and soybean meal.

Celluloses. Long fibrous cellulose derived from cotton (CF1 cellulose; catalog no. C-6663, lot no. 87F-0542; hereafter designated cellulose I, or I) was obtained from Sigma. Preparation of the other cellulose allomorphs is summarized in Fig. 1 and described in more detail below.

Cellulose II was prepared as follows. Sixty grams (= 57.4 g, dry weight) of cellulose I was suspended in 3 liters of rapidly stirred, ice-cold 20% (wt/vol) NaOH. After 1 h of incubation in an ice bath, the slurry was squeezed through a sheet of 30-μm-mesh Nitex nylon screen (catalog no. 3-30/21; Tetko, Inc., Elmsford, N.Y.). The retained solids were successively washed and resqueezed through the same sheet, using five water washes of ~0.5 liter each. The resulting cake was divided into three portions and placed into Imhoff settling cones that contained 1 liter of 1% (vol/vol) glacial acetic acid. The cellulose was allowed to settle, and the overlying liquid was removed by siphoning. Cones were refilled three times with 2% acetic acid, and the overlying liquid was removed by siphoning each time, following complete mixing and then settling of the cellulose. These washings were repeated 10 times, using distilled water as the resuspension agent. After the final settling, the celluloses were pooled, resuspended in 2 liters of 1% glacial acetic acid, stirred for 30 min, and then vacuum filtered through Whatman no. 1 filter paper. The cellulose cake was washed with 20 liters of distilled water (i.e., until the pH of the filtrate was the same as that of distilled water) and then secured inside nylon-reinforced paper towels and dialyzed against 8 liters of distilled water at 8°C; the bags were tightly squeezed between daily water changes to remove equilibrated liquid. After five dialysis cycles, the bags were lyophilized and the dried cellulose II powder was stored in airtight vials. Four separate batches were prepared (yield, 53 to 68%); these batches gave identical X-ray diffraction patterns characteristic of II and were put together into a single sample for use as a fermentation substrate and for preparation of cellulose III_{II} (see below).

Cellulose III_I was prepared from I by treatment with anhydrous ethylamine at 0°C for 4 h, as described by Segal et al. (26); residual ethylamine was removed under a stream of N_2 at room temperature, and the resulting product was placed in a vacuum oven at 40°C for 2 weeks. Treatment of II under the same reaction conditions failed to give more than ~5% conversion to the III_{II} allomorph. However, treatment of II with supercritical ammonia (135°C, 136 atm, 1 h [36]) resulted in >90% conversion to III (hereafter designated III_{II}).

Cellulose IV_I was prepared by heating 3- to 4-g batches of III_I in dry glycerol (200 g of glycerol per g of cellulose) at 260°C for 1 h under an N_2 sparge. After heating, the reaction mixtures were cooled to ~130°C and diluted with ~1 volume of dimethyl formamide. The mixtures were then vacuum filtered through a 0.22-μm nylon membrane (which required ~1 h, during which time the mixtures were held at ~90°C) and rinsed with 1 liter of methanol prior to drying overnight under vacuum at 40°C. The products (yield, 78 to 81%) from these preparations were mixed together for the fermentation experiments. Attempts to prepare cellulose IV_{II} from III_{II} by this treatment (at reaction times of up to 3 h) or by treatment with formamide at 120°C for 1 to 4 h (37) were not successful, resulting in minimal conversion.

Undegraded amorphous cellulose, prepared by treatment of I with paraformaldehyde-dimethyl sulfoxide-sodium methoxide (23), was used for most experiments. For one of the fermentation experiments, another batch of amorphous cellulose was prepared by ball milling I in a Spex mill for 4 h.

Physical properties of the cellulose allomorphs. Conversion to the desired allomorph was deduced (15) from powder X-ray diffraction spectra obtained by the method of Segal et al. (25) and from the known history of the samples. A relative crystallinity index was determined by acid hydrolysis kinetics (34). Particle sizes were determined by light microscopy, using a Carl Zeiss Axioskop microscope fitted with an ocular micrometer (Carl Zeiss, Oberkochen, Germany). Gross specific surface area (GSSA) was estimated from the particle size data, as described previously (32). The nitrogen contents of the cellulose preparations were determined from triplicate samples with a Carlo Erba NA1500 nitrogen analyzer (Fisons Instruments, Saddle Brook, N.J.).

Fermentation kinetics experiments. The allomorphs were subjected to in vitro fermentation, using either pure cultures of ruminal cellulolytic bacteria or mixed ruminal microflora. Each separate experiment was conducted with a single

inoculum tested against all of the cellulose preparations. The general procedures used for the fermentations were similar to those described previously (32), except that the fermentations were conducted on a smaller scale, in 50-ml serum vials. For experiments with pure cultures, vials contained 100 mg of cellulose, 8.0 ml of the modified Dehority's medium described above, 0.20 ml of 2.5% (wt/vol) cysteine HCl reducing agent, and 1.7 ml of inoculum. For experiments with the mixed ruminal microflora, vials contained 100 mg of cellulose, 8.0 ml of NH_4-supplemented McDougall buffer (32), 0.2 ml of 0.5% cysteine HCl–0.5% (wt/vol) $Na_2S \cdot 9H_2O$ reducing agent, and 1.8 ml of freshly collected and diluted ruminal fluid inoculum (32). Owing to changes in allomorphic form, crystallinity, and/or degree of polymerization which occur during sterilizing treatments (e.g., autoclaving [12] or gamma irradiation [5]), the experiments were conducted without sterilization of the substrate.

Cellulose was recovered and quantitated as described previously (32) except that the volume of neutral detergent solution was reduced to 20 ml. The weight loss data obtained by sacrificing paired vials of each cellulose substrate at each of six to eight different time points were fitted to a discontinuous first-order kinetic equation (16, 32) which yielded two kinetic parameters: a first-order rate constant (with units of $hour^{-1}$) and a discrete lag time (with units of hours).

Reversion of allomorphs. To determine whether incubation in the aqueous culture media caused reversion of the celluloses to other allomorphs, separate vials containing cellulose and fermentation media, but lacking the microbial inoculum, were incubated for 3 to 66 h under the same conditions as the inoculated vials. The contents of these control vials were vacuum filtered through 0.2-μm polycarbonate membranes, washed with ultrapure water, and dried under vacuum at 40°C. The resulting cellulose powders were then analyzed by X-ray diffraction as described above.

RESULTS

Properties of the cellulose allomorphs. The chemical treatments described above resulted in a series of cellulose preparations having X-ray diffraction patterns (Fig. 2) essentially identical to those of the different allomorphs described in the literature (15). Table 1 shows the particle size, surface area, relative crystallinity indices, and nitrogen contents of the different cellulose preparations. The allomorphs prepared in the laboratory showed a slight decrease in average fiber length, along with a slight decrystallization for III_I, III_{II}, and IV_I. Relatively little change was noted in GSSA (the gross surface area per unit mass of fiber), owing in part to the lower densities of the unit cells of the alternative allomorphs, which serve to elevate the GSSA relative to that of I.

The amorphous cellulose prepared by the paraformaldehyde-dimethyl sulfoxide-sodium methoxide method had a crystalline content of <10% (deduced from Fig. 2) and a considerably decreased average particle size (Table 1), which resulted in a fourfold-greater GSSA than that of the other materials.

As first noted by Segal et al. (26), cellulose III_I retained a considerable amount of nitrogen following its preparation with the ethylamine reagent. The nitrogen contents of the other preparations were minimal.

Reversion of cellulose allomorphs. All of the celluloses retained their allomorphic form upon incubation in the uninoculated fermentation medium for 66 h at 39°C, with the exception of III_I. This substrate showed approximately 20 to 30% reversion to I within a few hours of suspension in the culture medium; however, the extent of reversion did not increase upon longer incubation times.

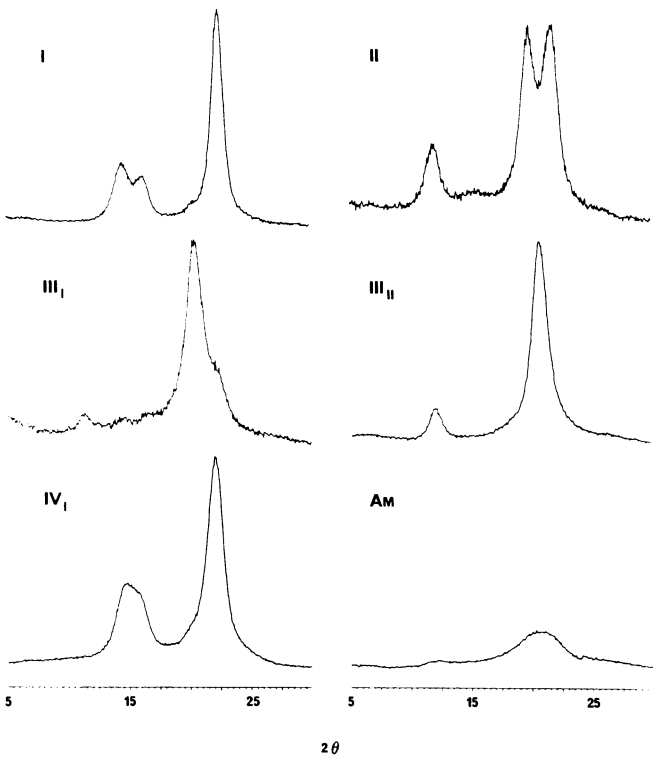

FIG. 2. Powder X-ray diffraction spectra of cellulose allomorphs. Am, amorphous cellulose prepared by the method of Schroeder et al. (23).

Fermentation kinetics. *R. flavefaciens* FD-1 fermented amorphous cellulose more rapidly than any of the other celluloses tested (Table 2). This strain digested I only slowly in batch culture, despite having been inoculated from a steady-state (i.e., exponential-growth-phase) chemostat culture growing on microcrystalline cellulose I. Other allomorphs of the cellulose I family (III_I and IV_I) were digested more rapidly than was I. III_{II} was degraded much more slowly than was III_I, and II was not degraded even after incubation for 3 weeks.

F. succinogenes S85 displayed a pattern of fermentation rates somewhat similar to that of *R. flavefaciens*. Amorphous cellulose was degraded most rapidly. Like *R. flavefaciens*, *F. succinogenes* degraded III_I, III_{II}, and IV_I more rapidly than it did I. In general, this organism also showed a greater ability to utilize the antiparallel celluloses: II was degraded, albeit slowly, and III_{II} was degraded almost as rapidly as III_I.

The kinetic profiles of the mixed ruminal microflora with the different celluloses displayed both similarities to and differences from those of the pure cultures. The inoculum from cow 748, like the pure cultures, degraded III_I and IV_I more rapidly than I, which in turn was degraded more rapidly than II. However, unlike the pure cultures, neither amorphous cellulose nor III_{II} was degraded any more rapidly than I. The inoculum from cow 507-J degraded I most rapidly of all of the substrates tested, while amorphous cellulose, III_I, and IV_I were degraded slightly more slowly and III_{II} and II were degraded even more slowly.

TABLE 1. Physical characteristics of cellulose allomorphs used in this study

Cellulose	Mean particle size[a]		% N	RCI[b]	D_{cr} (g/cm^3)[c]	Density (g/cm^3)[d]	GSSA (m^2/g)[e]
	Length (μm)	Width (μm)					
I[f]	182	20	<0.01	87.1	1.640	1.592	0.158
II	115.4	18.9	<0.01	90.9	1.613	1.582	0.145
III_I	143.6	19.3	2.38	70.4	1.546	1.464	0.151
III_{II}	113.0	18.2	0.08	71.3	1.546	1.467	0.162
IV_I	125.6	20.3	0.07	74.7	1.591	1.510	0.141
Amorphous	35.1	19.8	<0.01	<10		1.27	0.606

[a] Mean value of 300 measured particles.
[b] RCI, relative crystallinity index; determined by acid hydrolysis kinetics.
[c] Density of the crystalline regions, calculated by proportionality from the density and volume of the cellulose I unit cell (1.64 g/cm^3 and 0.656 nm^3, respectively; from the data of Woodcock and Sarko [35]) and the unit cell volumes of celluloses II (0.667 nm^3), III (0.696 nm^3), and IV (0.676 nm^3). Amorphous cellulose is assumed to have no crystalline regions.
[d] Estimated as: $\{[D_{cr} \times (RCI)] + [1.27 \times (100 - RCI)]\}/100$, where RCI is relative crystallinity index. The value of 1.27 g/cm^3 represents the density of amorphous cellulose. The calculation assumes a simple two-phase distribution of crystallinity, a considerable oversimplification of known distribution order (14).
[e] See reference 32; calculated as mean of 300 separate GSSA measurements.
[f] Data from Weimer et al. (32).

Visual examination of the celluloses recovered by the detergent fiber method from both mixed ruminal microflora fermentations revealed that amorphous cellulose (at all time points) and III_I (at 24 h and time points beyond) displayed a distinct yellowing characteristic of the cellulose fermentation of *R. flavefaciens*. Light microscopy and phase-contrast microscopy showed that all of the celluloses were virtually completely colonized with coccoid or coccobacillus bacterial forms at all time points in which cellulose degradation followed first-order kinetics (data not shown). Protozoan or fungal morphologies were not observed in fermentation vials incubated for 8 h or longer.

DISCUSSION

Structural features such as degree of crystallinity and available surface area are known to affect the digestion kinetics of cellulose by ruminal microorganisms (32). However, it is unlikely that the small differences among different allomorphic forms for these parameters (Table 1) can explain the large differences in digestion rate we observed for this set of substrates. In addition, peak widths on the X-ray diffractograms indicate that the different allomorphs have similar average crystallite sizes. This suggests that the different fermentation kinetics of these substrates result from more fundamental structural differences (e.g., allomorphic form).

Different cellulose allomorphs have both differences and similarities in certain unit cell structural features (Table 3). Our understanding of cellulose structure at the molecular level is based mostly on fiber diffraction studies in which stereochemical models are fit to X-ray or electron diffraction data. While these structures have high resolution, they are only as good as the models and their underlying diffraction data, which are themselves the subject of considerable controversy (1, 6, 7). We have elected to compare our fermentation kinetics data with the crystallographic structures elucidated by Sarko et al. (see Table 3 for references), since these structures (i) are based on plant (rather than algal or bacterial) celluloses; (ii) are as detailed as any currently available; (iii) have been developed by using internally consistent methods; and (iv) have enjoyed reasonably wide acceptance. The recent demonstration of two crystalline forms of cellulose I (designated Iα and Iβ; see references 2 and 29) have further complicated our concepts of cellulose structure. Plant celluloses (including the samples used in our experiments, which were derived from cotton) appear to be primarily Iβ (2), whose two-chain unit cell appears to have

TABLE 2. Normalized first-order rate constants and calculated discrete lag times for different celluloses during fermentation by pure and mixed cultures of ruminal microorganisms

Cellulose	Normalized rate constant[a]				Lag time (h)[b]			
	R. flavefaciens FD-1	*F. succinogenes* S85	Mixed ruminal microflora		*R. flavefaciens* FD-1	*F. succinogenes* S85	Mixed ruminal microflora	
			Cow 507-J	Cow 748			Cow 507-J	Cow 748
I	1.00 (0.13)	1.00 (0.06)	1.00 (0.06)	1.00 (0.05)	9.7 (2.7)	1.1 (0.2)	14.2 (0.1)	10.2 (0.4)
II	0	0.32 (0.06)	0.27 (0.01)	0.71 (0.09)	NA[c]	4.9 (1.4)	16.7 (0.1)	18.9 (1.6)
III_I	4.77 (0.06)	1.75 (0.17)	0.73 (0.02)	1.56 (0.01)	7.8 (0.5)	3.1 (0.8)	14.5 (0)	12.1 (0.5)
III_{II}	1.23 (0.19)	1.34 (0.17)	0.49 (0.01)	0.82 (0.10)	7.0 (0.5)	3.2 (1.7)	11.7 (0.1)	9.0 (1.1)
IV_I	4.08 (0.71)	2.20 (0.49)	0.89 (0.03)	2.02 (0.07)	10.5 (2.1)	2.7 (1.0)	10.3 (0.4)	9.3 (0.7)
Amorphous	9.58 (0.60)[d]	3.59 (0.39)[d]	0.84 (0.02)[d]	0.94 (0.01)[e]	5.7 (0.6)[d]	2.8 (1.7)[d]	4.8 (0.1)[d]	7.6 (0.2)[e]

[a] First-order rate constants were normalized to those of cellulose I. The actual rate constants for cellulose I in each experiment were as follows: *R. flavefaciens*, 0.0053 ± 0.0007 h^{-1}; *F. succinogenes*, 0.0271 ± 0.0016 h^{-1}; cow 507-J, 0.0890 ± 0.0059 h^{-1}; cow 748, 0.0574 ± 0.0032 h^{-1}. Coefficients of variation are given in parentheses.
[b] Coefficients of variation are given in parentheses.
[c] Not applicable (no degradation observed).
[d] Amorphous cellulose obtained by the method of Schroeder et al. (23).
[e] Amorphous cellulose obtained by ball milling.

TABLE 3. Comparison of some structural features of cellulose allomorphs, using the models of Sarko et al. (22)

Allomorph	Unit cell parameters[a]				Polarity of adjacent sheets	No. of hydrogen bonds[b]			O(6) hydroxyl rotation (°)[c]		Reference
	a	b	c	γ		Intrachain	Interchain	Intersheet	Corner chain	Center chain	
I	7.78	8.20	10.34	96.5	Parallel	8	4	0	180	180	35
II	9.09	7.96	10.31	117.3	Antiparallel	3	4	6	165	64	28
III_I	10.25	7.78	10.34	122.4	Parallel	8	4	0	173, 175	168, 164	22[d]
III_{II}	10.25	7.78	10.34	122.4	Antiparallel	6	4	2	167, 168	67, 66	22[d]
IV_I	8.03	8.13	10.34	90.0	Parallel	6	4	0	159, 140	163, 124	8

[a] a, b, and c, dimensions (in angstroms [1 Å = 0.1 nm]) along the *x*, *y*, and *z* axes, respectively, of the unit cell. γ, angle (in degrees) between a and b.
[b] Intrachain, within a single chain; interchain, between chains within the same sheet and excluding interchain bonds between chains in adjacent sheets; intersheet, interchain bonds between chains on adjacent sheets.
[c] Torsion angle of the O(5)-C(5)-C(6)-O(6) linkage, rounded to nearest degree.
[d] See models numbered 2 and 10b in indicated reference.

dimensions very similar to those of Sarko's cellulose I (Table 3).

All of the celluloses are composed of layered sheets thought to contain intrachain and interchain hydrogen bonding and to interact in some cases via hydrogen bonding between adjacent sheets (15, 21). Three of the celluloses (I and its interconvertible relatives III_I and IV_I) lack intersheet hydrogen bonds and are thought to display parallel sheet packing, while the other two (II and III_{II}) purportedly contain intersheet hydrogen bonds and display antiparallel sheet packing (22).

Interpretation of the fermentation kinetic data in terms of these structures permits identification of which structural features of the cellulose are most likely to determine its digestion kinetics by the ruminal microorganisms. The rapid degradation of amorphous cellulose by the pure cultures relative to the different crystalline allomorphs may be partly due to the fourfold-larger GSSA of the amorphous material (Table 1) (32). Both pure cultures also fermented III_I and IV_I considerably more rapidly than they did I. A slower fermentation of the cellulose II family (II and III_{II}) was indicated by the considerably lower degradation rate of III_{II} compared with that of III_I and by the very slow (*F. succinogenes*) or nonexistent (*R. flavefaciens*) degradation of II. Since the III_I and III_{II} preparations had nearly identical GSSAs and relative crystallinity indices and are thought to have identical unit cell dimensions, the differences in degradation kinetics between III_I and III_{II} may be due to the absence or presence, respectively, of the stabilizing effects of intersheet hydrogen bonds, perhaps also involving differences in parallel versus antiparallel chain packing schemes (21). The differences in the fermentation rates of III_I and III_{II} were probably even greater than are indicated by the above data, since the III_I preparation showed partial reversion to the more slowly degraded parent material (I) upon suspension in culture medium.

The enhanced degradation rate of both III_I and IV_I over that of I by both bacterial species may be due in part to reduced crystallinity (Table 1). In the case of III_I, the rate enhancement may also be a reflection of the skewed structure of the III_I lattice (22), which might expose alternating corner chain residues of the unit cell of III_I to the external environment (and thus to exoglucanases) to a greater extent than those of I. The enhanced degradation rate of IV_I over that of I by both bacterial species is surprising in view of the close similarities in chain packing schemes and unit cell dimensions proposed for these allomorphs. Crystallographic and modeling data suggest that I and IV_I differ primarily in the positions of the O(6) hydroxyl groups (8). In I, these groups are all nearly *tg*, while in IV_I, half of the center-chain O(6) hydroxyl groups are displaced approximately 20° to 55° from the *tg* position. However, since the O(6) hydroxyl groups of the crystalline celluloses are oriented in these positions only when buried within the crystallites (i.e., inaccessible to microbial cells or enzymes), it is unlikely that O(6) orientation can account for the substantial kinetic differences observed.

One structural feature not considered here is the lateral growth faces (i.e., the exposed planes) of the crystallite, which are distinct from the unit cell edges. These lateral faces have been characterized for algal (*Valonia*) cellulose, but not for higher plant cellulose (e.g., the cotton fibers from which the celluloses described here were obtained). Consequently, it is not possible for us to assess these lateral face effects on degradation kinetics.

The observation that amorphous cellulose and crystalline cellulose I display similar fermentation rates by mixed ruminal microflora is in accord with a previous study (32) which showed that degree of crystallinity is a relatively minor determinant of digestion rate by the total cellulolytic population within the rumen. However, amorphous cellulose showed a much shorter lag time before the onset of fermentation than did I, suggesting that at least some of the cellulolytic microorganisms recognize and/or attach to the amorphous material more rapidly than to crystalline cellulose.

Given the similarity in relative kinetic behavior of *F. succinogenes* and *R. flavefaciens* toward the different allomorphs, and the purported role of these species as major cellulolytic agents in the rumen (for reviews, see references 11, 27, and 31), it is surprising that the mixed ruminal microflora obtained from two different cows yielded kinetic profiles for the same series of allomorphs which differed considerably from each other and from the pure cultures. Several possible explanations for the disparity between the pure culture and mixed ruminal microflora data may be advanced. These could include (i) inherent differences in behavior of these species or their cellulolytic enzymes in pure culture compared with mixed culture; (ii) changes in the properties of these two pure cultures over many years of laboratory cultivation since their original isolation from the rumen; or (iii) the relative lack of importance of these species in the particular ruminal samples we examined. The data presented here do not permit evaluation of which of these possible explanations, if any, is responsible for the disparity between the pure-culture and mixed-culture data.

It is important to note that the various degradation rates reported here were determined by weight loss methods and do not necessarily imply differences in the rate of glycosidic bond cleavage. Because the cellulolytic enzymes cannot penetrate the crystalline lattice, it would seem that unit cell features not associated with the exposed surfaces of the cellulose fibril (e.g., intersheet hydrogen bonds) would not affect glycosidic bond cleavage, but may be important in the overall weight loss process by limiting the rate at which the chains or sheets delaminate from the cellulose fiber. Alternatively, differences in the thermodynamic stability of the different allomorphs may influence weight loss for similar reasons. These factors, even if nonbiological, could be important contributors to the overall process of cellulose biodegradation.

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